Carbohydrate Polymers 86 (2011) 684–690

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Effect of immunological enhancement of aloe polysaccharide on chickens immunized with *Bordetella avium* inactivated vaccine

Zhenhong Sun, Kai Wei, Zhengui Yan, Xiaolin Zhu, Xinjian Wang, Hui Wang, Yanling Tan, Pengcheng Sheng, Ruiliang Zhu*

College of Animal Science and Technology, Shandong Agricultural University, Taian, Shandong 271018, PR China

ARTICLE INFO

Article history:
Received 24 January 2011
Received in revised form 10 May 2011
Accepted 11 May 2011
Available online 19 May 2011

Keywords:
Aloe polysaccharide
Chicken
Bordetella avium (*B. avium*)
Antibody
Lymphocyte
Flow cytometry

ABSTRACT

This study was conducted to evaluate the effect of aloe polysaccharide on immune responses of chickens immunized with *Bordetella avium* (*B. avium*) vaccine. SPF chickens were randomly divided into 6 groups and vaccinated against *B. avium* vaccine which respectively contained aloe polysaccharide of three different dosages, propolis and pine pollen polysaccharide. The data showed that aloe polysaccharide significantly enhanced serum and bile antibody level, blood lymphocyte ratio and splenic T lymphocyte proliferation rate in groups I, IV and V. 40 mg/ml of aloe polysaccharide made the vaccine most effective and 20 mg/ml of propolis or 20 mg/ml of pine pollen polysaccharide achieved the same effect. These results suggested that aloe polysaccharide could significantly enhance immune effect of *B. avium* inactivated vaccine and had important implications for the further use of aloe polysaccharide as a new type of plant-derived immunopotentiator.

1. Introduction

Aloe barbadensis Miller (*Aloe vera*) is a tropical or subtropical plant with turgid lace-shaped green leaves. *Aloe vera* is rich in bioactive components such as aloe polysaccharide, anthraquinone compound, amino acid and microelement which endow it with a variety of pharmacological activities (Hamman, 2008; Rodríguez, Darias, & Díaz, 2010; Saada, Ussama, & Mahdy, 2003). Compared with aloe anthraquinone compound, aloe polysaccharide is an important chemical composition which represents a breakthrough in modern study on aloe biological activity. (Li, Luo, & Li, 2009; Xu et al., 2010). Many kinds of polysaccharides have been detected or isolated from aloe gel, including mannan, galactan, glucomannan, pectic substance, glucuronic acid-containing polysaccharide, etc. (Gauntt, Wood, McDaniel, & McAnalley, 2000; Liu, Wang, Xu, & Wang, 2007; Wang, Qi, & Liu, 2008). Like other plant polysaccharides, aloe polysaccharide has such functions as broad-spectrum immunoregulation, anti-radiation, anti-HIV, anti-ulcer, antioxidation and hypolipidemic and hypoglycemic effects (Lee et al., 2001). It acts not only on immune organs, but also can activate immunocytes, regulate the release of cytokine, promote the generation of antibody and enhance the immunological function of erythrocytes (Chen, Wu, Jiang, Huang, & Wang, 2005). As is shown by Liu et al. (2006), purified polysaccharide PAC-I was isolated from *Aloe vera* L. var. chinensis (Haw.) Berg. 0.5 ml of PAC-I (5 mg/ml) injected intraperitoneally into mice can elevate expression of macrophage activation markers (MHC-II and FcγR), promote phagocytosis of macrophages, and enhance tumor cell cytotoxic activity of macrophages in vivo.

Immune disfunction in livestock and poultry posed by bacterial and viral infection has already exerted extraordinarily adverse effects on livestock husbandry. Especially the long existence of the immunity inhibition diseases has created favorable conditions for the outbreak of poultry epidemic diseases. Chemical biosynthetic pharmaceutics (such as antibiotics) are overused, resulting in a sharp deterioration in meat quality and flavor. Research and development of new immunopotentiators is becoming the new focus of livestock husbandry research. Currently, a number of natural Chinese herbal immunopotentiators have been developed, achieving great efficacy in the realms of disease control and antibiotics residue resolution. Study on aloe polysaccharide mainly focuses on the development of human health products (Davis & Goux, 2009; Saini, Goyal, & Chaudhary, 2010). The application of aloe polysaccharide to poultry industry as immunopotentiator is rarely reported. Some scholars have already proved that the addition of aloe polysaccharide to chicken feeds can increase immune organ index and phagocytic index, and promote average daily intake

* Corresponding author at: Shandong Provincial Key Laboratory of Animal Biotechnology and Disease Control and Prevention, College of Animal Science and Technology, Shandong Agriculture University, No. 61 Daizong Road, Taian 271018, PR China. Tel.: +86 538 8242341.
E-mail addresses: zhurl@sdau.edu.cn, xhm.3333@163.com (R. Zhu).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.05.012

and average daily gain (Deng et al., 2008). In the light of relevant research of predecessors, our group have studied the effect of immunological enhancement of aloe polysaccharide on SPF chickens immunized with *B. avium* inactivated vaccine, which provides experimental evidence for the application of aloe polysaccharide as immunopotentiator.

2. Materials and methods

2.1. Materials

RPMI-1640 medium and fetal bovine serum were purchased from Gibco, USA. Concanavalin A (ConA; Sigma, USA), a T-cell mitogen, was diluted to a concentration of 0.025 mg/ml by RPMI-1640 medium. Propidium iodide (PI) dye was supplied by Amresco, USA. Lymphocytes separation medium was purchased from Cedarlane, Canada.

Rabbit anti-chicken antibody was acquired from Chinese Academy of Sciences. *B. avium* standard positive serum was preserved by microorganism research laboratory of Shandong Agricultural University. Propolis, purchased from Taian Experimental Bee Breading Apiary, was dissolved in anhydrous ethanol, and made into saturated propolis ethanol extract as propolis adjuvant. Pine pollen polysaccharide, with the purity of 72.2%, was extracted by hot water extraction and ethanol precipitation method and preserved by our laboratory (Wei et al., 2010).

2.2. Aloe polysaccharide

The optimized hot water extraction and ethanol precipitation method was adopted to extract aloe polysaccharide (Qi et al., 2008), and details of the method were as follows:

2–3 years old fresh leaves of *Aloe vera* were selected, washed and soaked in de-ionized water for about 30 min in order to eliminate the yellow juice exudated from the epidermis. Gel of the interior layer was cut up, homogenized and refrigerated overnight. Aloe juice and de-ionized water were mixed at the ratio of 1:10, extracted at 85 °C for 8 h, concentrated under reduced pressure, and centrifuged 3 times (12,000 r/min, 12 min, 15 °C) in order to remove all the impurities. The pH value of aloe juice concentrate was adjusted to 4.4. Ethanol with the final concentration of 80% was added to the aloe juice concentrate which was then precipitated for 8 h, and centrifuged at a high speed to produce polysaccharide precipitation. This precipitation was then washed successively using ethanol, acetone, and ether to remove protein and lipid. And finally via vacuum drying, aloe polysaccharide was obtained. Referring to the anthrone–sulphoacid method of Miao (2009), we used glucose standard curve to determine aloe polysaccharide content and worked out aloe polysaccharide yield rate and purity.

2.3. Preparation of B. avium inactivated vaccine

P8 strain of good cross-immunity and high virulence was selected from *B. avium* strains preserved in our laboratory for preparation of vaccine, inoculated in nutrient broth (glucose content 2%), and incubated in culture box at 37 °C for 24 h. Bacterium were then counted and centrifuged (5000 r/min, 10 min, 4 °C), supernatant removed. In the next step, bacterium was washed 3 times with PBS and suspended. An appropriate amount of glycerin and polyethylene glycol was added and thoroughly blended. This liquid was finally mixed and inactivated for 48 h by formaldehyde solution with the final concentration of 0.4%. *B. avium* vaccine, the final concentration of which was 5×10^9 CFU/ml, was prepared. Aloe polysaccharide, propolis and pine pollen polysaccharide were respectively added to equivalent *B. avium* inactivated vaccine. Then different *B. avium* inactivated vaccines with the aloe polysaccharide concentration of 40 mg/ml, 20 mg/ml and 10 mg/ml, propolis concentration of 20 mg/ml, and pine pollen polysaccharide concentration of 20 mg/ml were separately prepared.

2.4. Experimental chickens

210 1-day old chickens were selected (eggs brought from SPF chicken farm of Shandong Academy of Agricultural Sciences and hatched in our laboratory), fed under the same condition until 5-day old (average weight 25 g, *B. avium* maternal antibody 0), and randomly allotted into 6 groups.

Group division arrangement: chickens in groups I, II and III were respectively treated with cervical hypodermic injection of *B. avium* inactivated vaccine which contained 40 mg/ml, 20 mg/ml, 10 mg/ml of aloe polysaccharide; chickens in groups IV, V and VI were injected with propolis *B. avium* inactivated vaccine, pine pollen polysaccharide *B. avium* inactivated vaccine and *B. avium* inactivated vaccine. The injection dose given to each chicken in all groups was 1 ml. 5 chickens of each group were randomly sampled on days 3, 7, 14, 21, 28, 35, 42 post-vaccination. Relevant indexes were detected by methods as below.

2.5. Serum antibody titer detection

Serum antibody agglutination titers of *B. avium* were detected by microagglutination test.

B. avium diagnostic antigen: prepared at 4 °C, with reference to the method of Zhu, Zhang, and Tang (1991). Serum antibody agglutination titer was expressed as mean log 2.

2.6. Bile antibody level detection

5 chickens were randomly sampled from each group. Bile was extracted with syringe and centrifuged at 12,000 r/min for 10 min. Supernatant fluid was taken as specific antibody, and antibody levels were detected by indirect ELISA.

2.7. Blood lymphocyte ratio detection

5 chickens were randomly sampled from each group. 1 ml of aseptic blood was collected with EDTA anticoagulant tubes from heart. Blood lymphocyte ratios were detected by automatic blood cell analyzer.

2.8. Splenic T lymphocyte proliferation assay

Preparation of splenocyte suspension: spleen was taken under axenic condition, screened through a 200-mesh sieve, pulverized, cleaned, washed twice with PBS (penicillin–streptomycin content 100 IU/ml), mixed with lymphocytes separation medium, centrifuged at 1500 r/min for 10 min. Suspended lymphocytes were inhaled into centrifuge tube, washed twice with PBS, diluted with RPMI-1640 medium, counted, and adjusted to splenocyte suspension with a cell density of 1×10^6/ml.

Splenocyte suspension was separately moved into a 96-hole cell plate, 100 μl/hole. Then ConA (final concentration: 10 μg/ml) was added. At the same time, control holes with no addition of ConA were set. This plate was put into cell culture box for 48 h. After cultivation, the 6 splenocyte groups containing ConA and the splenocyte groups without ConA were respectively blended into suspension by blowing, through centrifugalization (1000 r/min, 10 min) and supernatant elimination, and were washed twice with PBS. 50 μg of PI dye was added to each tube after the adjustment to 1×10^6 cells per tube. They were placed in lucifugal environment for 30 min

and washed with PBS once again. Upon irradiation of the laser generated by flow cytometry (Model: Guaga Easy Cyte Mini) at the wavelength of 488 nm, lymphocyte percentages at S phase in both the experimental tubes and the control tubes were measured. Percentages at S phase (SPF) of the tubes containing ConA represented T lymphocyte proliferation degrees (Milovanova, 2007). SPF was calculated as under:

$$\mathrm{SPF} = \frac{\mathrm{S}}{(\mathrm{G0}+\mathrm{G1})+\mathrm{S}+(\mathrm{G2}+\mathrm{M})} \times 100\%$$

2.9. Statistical analysis

Data were expressed as mean ± SD. Duncan's multiple comparison analyse was performed using SPSS V14.0 software.

3. Results and discussion

3.1. Extraction of aloe polysaccharide

The yield rate of aloe polysaccharide extracted from aloe fresh leaves gel was 0.72%, and the purity of the gained aloe polysaccharide 82.4%.

Discrepancies in aloe polysaccharide extractions can cause differences in aloe polysaccharide contents, used doses and pharmacological effects (Davis & Goux, 2009; Xing & Li, 2009). In this study, 2–3 years old fresh leaves of *Aloe vera* were selected. Optimized hot water extraction and ethanol precipitation method was adopted to extract polysaccharide from gel. In the process of extraction, protein and lipid were eliminated to the maximum, and therefore the polysaccharide obtained in this assay had high purity with the doses used of high reference value.

3.2. Change of serum antibody agglutination titers

B. avium inactivated vaccine can stimulate B lymphocyte to proliferate. B lymphocyte then converts into plasmocyte, and secrete specific antibody. Agglutination reaction between *B. avium* and specific antibody can take place (Zhu et al., 1991). Changes of serum antibody agglutination titers can accurately and directly reflect the state of humoral immunity (Macpherson, Hunziker, McCoy, & Lamarre, 2001; Zhang, Wang, Hu, & Sun, 2009).

Serum antibody agglutination titers of chickens in all groups are presented in Table 1. Compared with the blank control group (VI), serum antibody agglutination titers in the aloe polysaccharide groups (I–III), the pine pollen polysaccharide group (V) and the propolis group (IV) were all higher than those in group VI. Among these groups, groups I and IV were significantly higher than group VI on days 7–35 post-vaccination ($P < 0.05$); group I and group IV were extremely significantly higher than group VI respectively on days 7–21 and days 14–21 post-vaccination ($P < 0.01$); group V was significantly higher than group VI on days 7–28 post-vaccination ($P < 0.05$). No significant differences were observed between groups II, III and group VI. Comparison among the 3 aloe polysaccharide groups showed, group I was significantly higher than groups II, III on days 7–35 post-vaccination ($P < 0.05$). Group II was higher than group III, with insignificant difference. Comparison between the aloe polysaccharide group and the propolis group showed, group I was higher than group IV in overall level, with insignificant difference; group II was significantly higher than group IV on days 7–28 post-vaccination ($P < 0.05$). Comparison between the aloe polysaccharide group and the pine pollen polysaccharide group showed, group I was higher than group V in overall level, with insignificant difference; groups II and III were significantly lower than group V on days 7–28 post-vaccination ($P < 0.05$).

Table 1
The effect of aloe polysaccharide on serum antibody agglutination titer in immunized chickens (log 2).

Group	Additive	Quantity (mg/ml)	Day post-vaccination (d)						
			3	7	14	21	28	35	42
I	Aloe polysaccharide	40	0.68 ± 0.83^{a}	3.74 ± 0.63^{CD}	6.72 ± 0.19^{F}	5.80 ± 0.54^{E}	5.38 ± 0.29^{DE}	4.56 ± 0.15^{d}	3.96 ± 0.46^{cd}
II	Aloe polysaccharide	20	0.50 ± 0.45^{a}	2.24 ± 0.13^{b}	4.28 ± 0.24^{d}	4.46 ± 0.28^{d}	3.38 ± 0.06^{c}	3.52 ± 0.34^{cd}	3.06 ± 0.19^{c}
III	Aloe polysaccharide	10	0.42 ± 0.03^{a}	2.00 ± 0.09^{b}	3.72 ± 0.60^{cd}	3.82 ± 0.15^{cd}	3.24 ± 0.09^{c}	3.18 ± 0.54^{c}	2.36 ± 0.28^{bc}
IV	Propolis	20	0.64 ± 0.21^{a}	3.28 ± 0.46^{c}	6.56 ± 0.25^{F}	5.80 ± 0.05^{E}	4.72 ± 0.25^{d}	4.20 ± 0.13^{d}	4.02 ± 0.37^{cd}
V	Pine pollen polysaccharide	20	0.46 ± 0.52^{a}	3.12 ± 0.38^{c}	6.06 ± 0.54^{ef}	5.52 ± 0.25^{e}	4.24 ± 0.34^{d}	3.84 ± 0.07^{cd}	3.64 ± 0.24^{cd}
VI	No additive	0	0.42 ± 0.02^{a}	1.61 ± 0.13^{b}	4.24 ± 0.17^{d}	3.80 ± 0.05^{d}	3.06 ± 0.09^{c}	2.60 ± 0.24^{c}	2.12 ± 0.30^{c}

Note: The data with different little letters show significant difference ($P < 0.05$). In comparison with group VI, the data with different capital letters in the same column show extremely significant difference ($P < 0.01$).

3.3. Change of bile antibody content

Local antibody content correlates positively with general immune level, and the level of bile antibody demonstrates local humoral immunity state (Yang, Zheng, Liu, & Ma, 2005). Bile antibody titers of chickens in all groups are presented in Table 2. Compared with the blank control group (VI), bile antibody contents in groups I–V were all higher than those in group VI. Among these groups, significant differences were observed between groups I, IV, V and group VI on day 7 post-vaccination ($P < 0.05$); group I was extremely significantly higher than group VI on days 21–28 post-vaccination ($P < 0.01$); group IV was extremely significantly higher than group VI on day 21 post-vaccination ($P < 0.01$). No significant differences were observed between groups II, III and group VI. Comparison among the 3 aloe polysaccharide groups showed, group I was higher than group II and group III respectively on days 21–35 and days 7–35 post-vaccination ($P < 0.05$); group II was higher than group III in overall level, with insignificant difference. Comparison between the aloe polysaccharide groups and the propolis group showed, group I was higher than group IV in overall level, with insignificant difference; group II was significantly lower than group IV on days 21–35 post-vaccination ($P < 0.05$); group III was significantly lower than group IV on days 7–35 post-vaccination ($P < 0.05$). Comparison between the aloe polysaccharide groups and the pine pollen polysaccharide group showed, group I was higher than group V in overall level, with insignificant difference; group II was lower than group V, and this difference was significant on days 21–35 post-vaccination ($P < 0.05$); group III was significantly lower than group V on days 7–35 post-vaccination ($P < 0.05$).

3.4. Change of blood lymphocyte ratios

Lymphocyte ratio is the percentage of lymphocytes in the total count of leucocytes. *B. avium* inactivated vaccine can induce specific proliferation of lymphocytes (including B cells and helper T cells) within the body (Peltola, Mertsola, & Ruuskanen, 2006). Change of blood lymphocyte ratios can reflect the state of general immunity. Automatic blood cell analyzer was used in automatic separating, selecting and counting, which is more accurate and speedy than conventional microscopic counting.

Blood lymphocyte ratios of chickens in all groups are presented in Table 3. Compared with the blank control group (VI), the overall blood lymphocyte ratios in groups I–IV and group V were all higher than those in group VI. Among these groups, groups I and IV were extremely significantly higher than group VI, respectively, on days 7–28 and days 14–21 post-vaccination ($P < 0.01$); group V was significantly higher than group VI on days 7–28 post-vaccination ($P < 0.05$); no significant differences were observed between groups II, III and group VI. Comparison among the 3 aloe polysaccharide groups showed, group I was higher than group II and group III, and this difference was significant on days 14–35 post-vaccination ($P < 0.05$); group II was higher than group III, with insignificant difference. Comparison between the aloe polysaccharide groups and the propolis group showed, group I was higher than group IV in overall level, with insignificant difference; groups II, III was lower than group IV, and this difference was significant on days 14–35 post-vaccination ($P < 0.05$). Comparison between the aloe polysaccharide group and the pine pollen polysaccharide group showed, group I was higher than V, with insignificant difference; groups II, III were lower than V, and the difference was significant on days 14–21 post-vaccination ($P < 0.05$).

3.5. Change of splenic T lymphocyte proliferation rates

Splenic T lymphocyte proliferation rates of chickens in all groups are presented in Table 4. Compared with the blank con-

Table 2
The effect of aloe polysaccharide on bile antibody content in immunized chickens (490 nm, OD).

Group	Additive	Quantity (mg/ml)	Day post-vaccination (d)					
			3	7	14	21	28	35	42
I	Aloe polysaccharide	40	0.229 ± 0.016^{ab}	0.478 ± 0.024^{c}	0.613 ± 0.050^{d}	0.876 ± 0.105^{F}	0.761 ± 0.100^{EF}	0.614 ± 0.011^{d}	0.556 ± 0.006^{CD}
II	Aloe polysaccharide	20	0.115 ± 0.008^{a}	0.381 ± 0.013^{bc}	0.525 ± 0.106^{cd}	0.649 ± 0.030^{d}	0.534 ± 0.046^{cd}	0.464 ± 0.024^{c}	0.424 ± 0.004^{c}
III	Aloe polysaccharide	10	0.105 ± 0.011^{a}	0.308 ± 0.009^{b}	0.439 ± 0.001^{c}	0.631 ± 0.024^{d}	0.510 ± 0.110^{cd}	0.461 ± 0.031^{c}	0.440 ± 0.100^{c}
IV	Propolis	20	0.226 ± 0.026^{ab}	0.469 ± 0.007^{c}	0.600 ± 0.021^{d}	0.826 ± 0.019^{f}	0.721 ± 0.102^{E}	0.603 ± 0.111^{d}	0.534 ± 0.101^{cd}
V	Pine pollen polysaccharide	20	0.132 ± 0.005^{a}	0.451 ± 0.106^{c}	0.576 ± 0.040^{d}	0.785 ± 0.016^{ef}	0.709 ± 0.006^{e}	0.565 ± 0.124^{d}	0.509 ± 0.021^{cd}
VI	No additive	0	0.090 ± 0.020^{a}	0.299 ± 0.017^{b}	0.426 ± 0.002^{c}	0.643 ± 0.10^{d}	0.509 ± 0.014^{c}	0.441 ± 0.022^{c}	0.331 ± 0.001^{b}

Note: The data with different little letters show significant difference ($P < 0.05$). In comparison with group VI, the data with different capital letters in the same column show extremely significant difference ($P < 0.01$).

Table 3
The effect of aloe polysaccharide on blood lymphocyte ratio in immunized chickens (%).

Group	Additive	Quantity (mg/ml)	Day post-vaccination (d)						
			3	7	14	21	28	35	42
I	Aloe polysaccharide	40	72.64 ± 0.79^{b}	79.28 ± 1.42^{d}	86.76 ± 2.17^{F}	85.96 ± 0.88^{F}	82.38 ± 3.21^{E}	81.98 ± 1.36^{E}	76.54 ± 0.55^{cd}
II	Aloe polysaccharide	20	71.28 ± 3.62^{ab}	76.18 ± 0.05^{cd}	83.32 ± 1.39^{e}	80.12 ± 0.67^{d}	78.86 ± 1.22^{d}	78.48 ± 1.09^{d}	74.50 ± 0.29^{c}
III	Aloe polysaccharide	10	70.92 ± 0.49^{a}	76.04 ± 1.69^{cd}	83.54 ± 0.57^{e}	80.01 ± 1.26^{d}	78.48 ± 2.05^{d}	76.90 ± 1.24^{cd}	73.52 ± 0.20^{bc}
IV	Propolis	20	72.50 ± 1.24^{b}	78.76 ± 0.53^{d}	86.46 ± 0.42^{F}	84.84 ± 0.53^{EF}	82.38 ± 2.45^{E}	81.76 ± 0.67^{E}	76.50 ± 0.83^{cd}
V	Pine pollen polysaccharide	20	72.10 ± 0.67^{ab}	78.38 ± 1.62^{d}	85.86 ± 2.49^{f}	84.46 ± 1.42^{ef}	81.08 ± 0.94^{de}	79.42 ± 0.95^{d}	73.98 ± 0.57^{bc}
VI	No additive	0	68.02 ± 0.81^{a}	75.02 ± 1.16^{c}	82.82 ± 1.29^{e}	79.74 ± 1.06^{d}	78.32 ± 0.48^{d}	76.62 ± 2.11^{cd}	73.34 ± 0.37^{bc}

Note: The data with different little letters show significant difference ($P < 0.05$). In comparison with group VI, the data with different capital letters in the same column show extremely significant difference ($P < 0.01$).

Table 4
The effect of aloe polysaccharide on splenic T lymphocyte proliferation in immunized chickens (%).

Group	Additive	Quantity (mg/ml)	Day post-vaccination (d)						
			3	7	14	21	28	35	42
I	Aloe polysaccharide	40	38.76 ± 3.46^{ab}	47.82 ± 1.94^{cd}	56.87 ± 1.49^{EF}	59.28 ± 3.07^{F}	61.52 ± 2.65^{F}	58.73 ± 2.54^{f}	54.48 ± 1.35^{e}
II	Aloe polysaccharide	20	35.12 ± 1.20^{a}	42.95 ± 2.16^{b}	47.69 ± 2.11^{cd}	50.83 ± 2.34^{d}	54.67 ± 1.81^{e}	52.41 ± 1.91^{de}	49.06 ± 1.82^{d}
III	Aloe polysaccharide	10	35.09 ± 1.02^{a}	42.10 ± 2.64^{b}	47.01 ± 0.85^{cd}	49.15 ± 1.22^{d}	52.00 ± 2.21^{de}	52.13 ± 1.64^{de}	48.68 ± 1.32^{d}
IV	Propolis	20	38.49 ± 2.08^{ab}	47.68 ± 5.26^{cd}	54.62 ± 1.06^{E}	58.53 ± 1.71^{F}	61.47 ± 1.03^{F}	58.21 ± 2.03^{f}	54.73 ± 1.77^{e}
V	Pine pollen polysaccharide	20	38.25 ± 1.07^{ab}	46.09 ± 3.16^{cd}	53.73 ± 3.01^{de}	56.38 ± 3.20^{ef}	60.68 ± 2.24^{F}	57.82 ± 1.49^{f}	54.18 ± 1.59^{e}
VI	No additive	0	35.07 ± 2.61^{a}	43.75 ± 0.69^{bc}	45.13 ± 1.19^{c}	48.44 ± 2.02^{cd}	52.92 ± 1.09^{de}	52.06 ± 2.67^{de}	49.38 ± 0.61^{d}

Note: The data with different little letters show significant difference ($P < 0.05$). In comparison with group VI, the data with different capital letters in the same column show extremely significant difference ($P < 0.01$).

trol group (VI), splenic T lymphocyte proliferation rates in groups I–V were all higher than those in group VI. Significant difference was observed between groups I, IV, V and group VI on days 14–42 post-vaccination ($P < 0.05$), among which groups I and IV were extremely significantly higher than group VI on days 14–28 post-vaccination ($P < 0.01$); group V is extremely significantly higher than group VI on days 28 post-vaccination ($P < 0.01$); no significant difference was observed between groups II, III and group VI. Comparison among the 3 aloe polysaccharide groups showed, from day 7 post-vaccination, group I was significantly higher than groups II, III ($P < 0.05$); group II was higher than group III in overall level, with insignificant difference. Comparison between the aloe polysaccharide groups and the propolis group showed, group I was slightly higher than group IV in overall level, with insignificant difference; groups II, III were both lower than group IV, and this difference was significant from day 7 post-vaccination ($P < 0.05$). Comparison between the aloe polysaccharide group and the pine pollen polysaccharide group showed, group I was slightly higher than group V in overall level, with insignificant differences; groups II and III were both lower than group V, and this difference was significant from day 21 post-vaccination.

Splenic T lymphocyte proliferation rate is the most direct index reflecting cellular immunity (Li, Santoso, & Lo, 2007). ConA is the nonspecific stimulus of T lymphocyte proliferation. Other cells not subjected to stimulation will gradually undergo apoptosis process during cultivation. Their incomplete cytomembrane enables cell nucleus to be dyed by PI dye. Upon the irradiation of the laser generated by flow cytometry at the wavelength of 488 nm, lymphocyte percentages at DNA proliferative phase (S phase) were measured, so as to reflect lymphocyte proliferative degrees (Babetta, 2009; Zhang et al., 2010). This method excluded divergence caused by unequal cell numbers, and was quick and reliable.

Judging from the design of experiment, this study is of important practical significance. Our group has reported that pine pollen polysaccharide can elevate immune indices of normal mice and immunosuppressed mice, and the injection dose of 400 mg/kg has the highest efficiency (Wei et al., 2010). Propolis is not only the natural efficient immunostimulant, but also the excellent immunoadjuvant (Jin et al., 2009; Ramanauskiene, Inkeniene, Savickas, Masteikova, & Brusokas, 2009). Based on relevant experiment achievements, this study drew on effective doses of pine pollen polysaccharide and propolis and designed 3 different doses of aloe polysaccharide separately as the high, medium and low dose, so as to explore the effect of immunological enhancement of aloe polysaccharide on immunized chickens. At the same time, we set the pine pollen polysaccharide control group and the propolis control group, which facilitated the evaluation of the actual effects of aloe polysaccharide. Based on an overall consideration of many factors such as immunological function, material source, and extraction cost of the 3 additives, our group hold that aloe polysaccharide possesses fairly good economic and practical value, and is more fit to be applied as an excellent immunopotentiator. Furthermore, compared with chemical synthetic pharmaceuticals, it has positive significance in enhancement of immunity, prevention of immunosuppressive diseases and elevation of animal products quality. For these reasons aloe polysaccharide has higher clinical application value.

4. Conclusions

The optimized hot water extraction and ethanol precipitation method was adopted to extract aloe polysaccharide. The yield rate was 0.72%, and the purity degree 82.4%. Any dose of aloe polysaccharide could improve serum and bile antibody level, blood lymphocyte ratio and splenic lymphocyte proliferation rate of the chickens immunized with *B. avium* inactivated vaccine, and the dose of 40 mg/ml had the most obvious efficacy, which differed insignificantly from the immunological enhancing effect of 20 mg/ml of propolis or 20 mg/ml of pine pollen polysaccharide. Aloe polysaccharide had a significant enhancing effect on immunological function of *B. avium* inactivated vaccine. It is fit to be applied as an excellent immunopotentiator.

Acknowledgments

This study was funded by National Natural Science Foundation of China (30972183, 30740077) and Specific Technology Development Projects of Taian (20103001).

References

Babetta, L. M. (2009). Flow cytometry: A multipurpose technology for a wide spectrum of global biosecurity applications. *Journal of the Association for Laboratory Automation*, *14*(3), 148–156.

Chen, X. D., Wu, B. Y., Jiang, Q., Huang, L. Y., & Wang, Z. C. (2005). Effect of *Aloe coarse* polysaccharide on cytokine secretion of keratinocytes in vitro. *China Journal of Chinese Materia Medica*, *30*(24), 1944–1946.

Davis, B., & Goux, W. J. (2009). Single-laboratory validation of an NMR method for the determination of *Aloe vera* polysaccharide in pharmaceutical formulations. *Journal of AOAC International*, *92*(6), 1607–1616.

Deng, Y. Y., Wu, C. R., Hu, F. P., Yang, X. L., Zuo, J. B., & Zeng, X. J. (2008). Effect of aloe polysaccharide on immune organ in senile mice. *Journal of TCM University of Hunan*, *28*(2), 25–27.

Gauntt, C. J., Wood, H. J., McDaniel, H. R., & McAnalley, B. H. (2000). Aloe polymannose enhances anti-coxsackievirus antibody titers in mice. *Phytotherapy Research*, *14*(4), 261–265.

Hamman, J. H. (2008). Composition and applications of *Aloe vera* leaf gel. *Molecules*, *13*(8), 1599–1616.

Jin, E. G., Chen, J., Qian, Y. G., Chen, C., Zeng, X. H., Gong, S. Y., et al. (2009). Study on the effect of *Astragalus* polysaccharide adjuvant to inactivated vaccine of *Riemerella anatipestifer* serotype I. *China Animal Husbandry & Veterinary Medicine*, *36*(3), 151–153.

Lee, J. K., Lee, M. K., Yun, Y. P., Kim, Y., Kim, J. S., Kim, Y. S., et al. (2001). Acemannan purified from *Aloe vera* induces phenotypic and functional maturation of immature dendritic cells. *International Immunopharmacology*, *1*(7), 1275–1284.

Li, T. D., Luo, Y., & Li, J. G. (2009). Research progress on biological activity of *Aloe vera* L. polysaccharide. *Journal of Anhui Agricultural Sciences*, *37*(5), 2033–2035.

Li, C. H., Santoso, S., & Lo, D. D. (2007). Quantitative analysis of T cell homeostatic proliferation. *Cellular Immunology*, *250*, 45–54.

Liu, C., Leung, M. Y. K., Koon, J. C. M., Zhu, L. F., Hui, Y. Z., Yu, B., et al. (2006). Macrophage activation by polysaccharide biological response modifier isolated from *Aloe vera* L. var. chinensis (Haw.) Berg. *International Immunopharmacology*, *6*(11), 1634–1641.

Liu, C. H., Wang, C. H., Xu, Z. L., & Wang, Y. (2007). Isolation, chemical characterization and antioxidant activities of two polysaccharides from the gel and the skin of *Aloe barbadensis* Miller irrigated with sea water. *Process Biochemistry*, *42*, 961–970.

Macpherson, A. J., Hunziker, L., McCoy, K., & Lamarre, A. (2001). IgA responses in the intestinal mucosa against pathogenic and non-pathogenic microorganisms. *Microbes and Infection*, *3*(12), 1021–1035.

Miao, Y. Y. (2009). Extraction and determination of polysaccharides from *Aloe barbadensis* Miller. *Chinese Archives of Traditional Chinese Medicine*, *27*(10), 2172–2175.

Milovanova, T. N. (2007). Comparative analysis between CFSE flow cytometric and tritiated thymidine incorporation tests for beryllium sensitivity. *Cytometry Part B – Clinical Cytometry*, *72*(4), 265–275.

Peltola, V., Mertsola, J., & Ruuskanen, O. (2006). Comparison of total white blood cell count and serum C-reactive protein levels in confirmed bacterial and viral infections. *The Journal of Pediatrics*, *149*(5), 721–724.

Qi, X. M., Wang, J., Hou, B., Wang, J. L., Mon, L. L., & Feng, C. (2008). Optimize and search on process for extraction of *Aloe* polysaccharides. *Food Research and Development*, *28*(4), 25–27.

Ramanauskiene, K., Inkeniene, A. M., Savickas, A., Masteikova, R., & Brusokas, V. (2009). Analysis of the antimicrobial activity of propolis and lysozyme in semisolid emulsion systems. *Acta Poloniae Pharmaceutica*, *66*(6), 681–688.

Rodríguez, E., Darias, M. J., & Díaz, R. C. (2010). *Aloe vera* as a functional ingredient in foods. *Critical Reviews in Food Science and Nutrition*, *50*(4), 305–326.

Saada, H. N., Ussama, Z. S., & Mahdy, A. M. (2003). Effectiveness of *Aloe vera* on the antioxidant status of different tissues in irradiated rats. *Pharmazie*, *58*(12), 929.

Saini, M., Goyal, P. K., & Chaudhary, G. (2010). Anti-tumor activity of *Aloe vera* against DMBA/croton oil-induced skin papillomagenesis in Swiss albino mice. *Journal of Environmental Pathology Toxicology and Oncology*, *29*(2), 127–135.

Wang, Y. L., Qi, J. S., & Liu, Q. Y. (2008). Determination of aloin and *Aloe* polysaccharides in American *Aloe vulgaris* in the three gorges drainage basin. *Journal of Chongqing Three Gorges University*, *24*(110), 1–3.

Wei, K., Zhu, R. L., Sun, Z. H., Tan, Y. L., Wang, H., Wang, X. J., et al. (2010). Research on the effect of immune enhancement of Taishan pine pollen polysaccharide in murine. *Scientia Agricultura Sinica*, *43*(17), 3645–3652.

Xing, J. M., & Li, F. F. (2009). Purification of aloe polysaccharides by using aqueous two-phase extraction with desalination. *Natural Product Research*, *23*(15), 1424–1430.

Xu, C., Ruan, X. M., Li, H. S., Guo, B. X., Ren, X. D., Shuang, J. L., et al. (2010). Anti-adhesive effect of an acidic polysaccharide from *Aloe vera* L. var. *chinensis* (Haw.) Berger on the binding of *Helicobacter pylori* to the MKN-45 cell line. *Journal of Pharmacy and Pharmacology*, *62*(12), 1753–1759.

Yang, Y. R., Zheng, S. M., Liu, J., & Ma, C. Q. (2005). Dynamic changes of the relative content of immunoglobulins in local humor and immune organs index of chicks administrated with probiotics. *Acta Veterinaria et Zootechnica Sinica*, *36*(4), 352–356.

Zhang, N. W., Li, J. F., Hu, Y. X., Cheng, G. L., Zhu, X. Y., & Liu, F. Q. (2010). Effects of astragalus polysaccharide on the immune response to foot-and-mouth disease vaccine in mice. *Carbohydrate Polymers*, *82*, 680–686.

Zhang, X. H., Wang, D. Y., Hu, Y. L., & Sun, J. L. (2009). Immunological enhancement of astragalus polysaccharide (APS) on the humoral immunity of chicken. *Chinese Journal of Veterinary Science*, *29*(3), 312–314.

Zhu, R. L., Zhang, S. X., & Tang, K. X. (1991). Primary study on the disease caused by *Bordetella avium* in chickens. *Journal of Shandong Agricultural University*, *22*(1), 92–94.